Proposed Particulate Matter Toxicity Studies

LT Erin Wilfong NHRC/EHEL
erin.wilfong@wpafb.af.mil
937-904-9474
Dr. Jeff Gearhart AFRL/HEPB

- Military personnel have emphasized concern regarding air quality due to the high levels of dust generated from blowing desert sand and the movement of troops and equipment *(LLRI, 2003)*
  - Numerous metals (Ca, Si, Fe, Al, Mg, K, Ni, Mn, Cr) and organisms (diphtheroids, streptococci ect) have been detected in Middle East sand *(Perdue et al., 1991)*
  - Additional contaminants as a result of military operations (Jet fuel, pesticides, combustion byproducts, ect)
  - Sand listed as a potential adverse substance that when combined with other experiences/exposures in a wartime environment may contribute to Gulf War Illness *(NIH, 1994)*

- **USACHPPM fact sheet "Dust and Sand"**
  - Persian Gulf Region: fine clay dust and sand
  - Significant amount in respirable size range (PM_{10})
  - *Acute*: cold/flu like symptoms including cough, runny nose, eye and throat irritation and shortness of breath
  - *Chronic*: PGW studies inconclusive. Some increases in asthma suggested.
  - Symptoms due to PM and not silica content
    - Silica is below harmful levels in the sand
  - http://chppm-www.apgea.army.mil/documents/FACT/65-037-0503.pdf

- **NEHC "Environmental Exposures Particulates"**
  - Sand and industrial/vehicular contributions comprise majority of PM
  - Cough, increased sputum, worsening of pre-existing asthma.
  - Persistent effects are not anticipated but are possible
  - http://www.pdhealth.mil/deployments/gulfwar/enviro_part.asp
  - http://chppm-www.apgea.army.mil/usachppmresources/ParticulateMatterFinal-26Nov02.pdf

- **Navy Bureau of Medicine & Surgery**
  - "Silicosis and Operational Exposures to Dust and Sand" NEHC-36 14 Nov 1990
  - Issue: Are military personnel at risk for silicosis or other pulmonary diseases due to exposure to sand and dust?
  - Concluded there was no risk for silicosis to military personnel
    - No reports
    - WWII Armored Medical Research Laboratory
      - Dust exposures: negligible hazard

- **Desert Lung Syndrome**
  - Deposition of silica containing dust in the lungs reported in inhabitants of the Saharan, Libyan, Negev and Arabian Deserts
  - After years of exposure develop a benign, non-progressive pneumoconiosis referred to as "Desert Lung Syndrome"
    - Bar-Ziv and Goldberg (1974) *Arch Environ Health*
    - Not Occupational Silicosis
      - Asymptomatic
      - Does not progress over time
    - Sand is "old" vs. "freshly-fractured" particles in occupational setting

- **Pulmonary Alveolar Microlithiasis (PAM)**
  - Characterized by progressive formation of intra-alveolar calcified microgranules; cause unknown
  - Disease progression may extend over 20 yrs
  - May be asymptomatic or have a chronic cough
  - Cases reported in Egypt, Saudi Arabia, Turkey
    - Al-Kubaisi et al. (2000) Case reports of PAM in Iraq
  - Possible causes?
    - Sand particles *(Biary et al 1993 and Felson 1988)*

- **Desert Storm Pneumonitis (Al Eskan Disease)**
  - Operation Desert Shield in the Al Eskan, Saudi Arabia (1991).
  - Acute desert-related respiratory tract infection
  - Symptoms:
    - Conjunctiva and nasal mucosa congested
    - Irritating dry cough
    - Fever, sore throat, malaise (varied)

(Kornyi-Both et al. (1992) Mil. Med. 9:452-461)

- Was the sand pathogenic??
  - Grains heavily agglomerated
  - Non-agglomerated grains ranged from 0.1 μm to 0.25 μm
  - Ca, Si, Mg, Al, Fe
    - Ca 5x higher than Si
  - Culture results
    - *Asperigillus niger, Chrysosporium & Acremonium* species, *Cryptococcus albiolus*

- Concluded: Al Eskan disease is a hyperergic lung condition
  - Alveolar macrophages overwhelmed by sand
  - ↓ resistance to infectious agents (i.e. bird droppings, viral, bacterial agents)

- **Respiratory disease during Operation Desert Shield**
  - Epidemiologic survey
    - 2598 male ground troops; 4 units in NE Saudi Arabia
    - Nov 1990- Jan 1991; mean stay 102 days
  - Survey questions
    - Respiratory complaints
      - Sore throat 34.4%
      - Cough 43.1%
      - Persistent rhinorrhea 15.4%
      - Not able to work 8.1%
    - Sleeping conditions
  - Sand analysis (surface samples)
    - crystallized silica, Ca carbonates, silicates of Al
    - 0.21% was respirable (<10 μm)

- **Conclusion**
  - The frequent upper respiratory complaints among Operation Desert Shield troops were found to be related to both housing conditions and exposure to the outside environment

(Richards et al., (1993) Am. J Public Health 83: 1326-1329)

- **Acute Eosinophilic Pneumonia**
  - 18 cases in military personnel deployed to Iraq between 3/03-3/04
    - 2 deaths
    - 4 additional cases since 3/04
    - febrile illness, respiratory symptoms (cough, dyspnea)
    - evidence of alveolar & interstitial infiltrates on chest radiograph
    - peaked in the summer months
    - 100% of patients smoked tobacco and 94% reported exposure to fine airborne sand or dust
  - Etiology unclear

(Shorr et al., (2005) JAMA 292: 2997-3005.)

- **Severe Acute Pneumonitis**
  - 19 cases in military personnel deployed to CENTCOM
    - 2 deaths
    - bilateral pneumonitis requiring intubations and mechanical ventilation
    - 4 had evidence of a microbial infection
    - 10 diagnosed as AEP
    - Majority (79%) smoked and were exposed to heat, dust and various environmental pollutants
  - Etiology unclear

(MMWR Sept 12, 2003, 52:857-859)

- ***In Vivo* Studies**
  - (Perdue et al., 1992) “Surgical Significance of Persian Gulf Sand”
  - Sampled desert sand from multiple sites in Saudi Arabia during Operation Desert Shield (1990)
    - > 85% smaller than 10 μm
    - Ca>Si>Fe>Al, K>Mg & bacteria
  - 0.100 g of sand suspended in 1.5 cc PBS
    - 10 rats injected (i.p.)
    - 6 rats injected (i.p.) supernatant only
  - Histology of intraperitoneal organs
    - granulomatous, peritonitis, serositis and inflammation (rats injected with suspension)
    - inflammation (rats injected with supernatant)
  - Clinical signs of systemic inflammation

- ***In Vitro* Studies**
  - Geng et al., (2005) *Tox Letters* 157:129-137
  - Respiratory toxicity of blowing sand $PM_{2.5}$ unknown
  - $PM_{2.5}$ high volume air sampler
    - Blowing sand days (airborne $PM_{2.5}$ 191 ± 70 $\mu g/m^3$)
    - Non-blowing sand days (airborne $PM_{2.5}$ 59.8 ± 29.3 $\mu g/m^3$)
  - Wuwei city, Gansu Providence, China
    - Bordered by the Badian Jaran and Tengger Deserts
  - Rat AM "first responders" for inhaled particles
    - 4 hr exposures (0, 33, 100 or 300 µg/ml)

- ***In Vitro* Studies**
  - Decreased cell viability $\geq$ 150 µg/ml
  - Plasma membrane permeability
    - Significant increase in LDH and ACP at 300 µg/ml
  - Significant increase in intracellular Ca^{+2} at 100 and 300 µg/ml
  - Significant decrease in GSH at 300 µg/ml

- Conclusion
  - Sand $PM_{2.5}$ can damage AM and cause cytotoxicity/cell death
  - No statistically significant difference between normal and blowing $PM_{2.5}$

- ***In Vitro* Studies**
  - Kim et al., (2003) *J. Biosci.* 28:77-81
  - Yellow sand collected in Gunsu Province of China (surface)
    - Particle size < 10 μm
    - Si > Al > Ca > Fe > K > Na, Mg
    - Irradiated for sterilization
  - Exposed rat alveolar type II cells
    - up to 24 hr exposures to saline, sand, silica (+ control) or TiO_2 (inert)
    - Doses: 100 μg/cm^2 , 1mg/cm^2 and 5 mg/cm^2

- **Results**
  - Sand and silica significantly decreased viability at 100 μg/cm^2
  - Intracellular calcium was significantly increase following sand or silica exposure (5 mg/cm^2)
  - TNF-α increased following sand or silica exposure (100 μg/cm^2)
  - Fenton activity was increased following sand or silica exposure (100 μg/cm^2)
    - Sand had a stronger (2x) fenton activity than silica
  - All particles generated H_2O_2 and nitrate

- ***In Vitro* Studies**
  - Huang et al., 2004 “Impact of dust story $PM_{2.5}$ and PM10 on the phagocytic function of AM of rat”
  - $PM_{2.5}$ and PM_{10} collected during a dust storm in urban Beijing, China
  - Exposed rat AM
    - Dose dependent impairment of phagocytic function ($\geq$ 20 µg/ml)

- Purpose of proposed studies
  - To determine if a single exposure to Iraqi PM (sand) causes airway inflammation and injury
  - Samples collected from Pad 15 in Iraq
    - PM_{10}
    - PM_{20}
    - PM_{40}

- Phase I
  - *In vitro* studies
    - Lung stimulant fluids will be used to determine the extent and rate at which the different sized sand particles would release metals and other chemicals constituents in the respiratory tract of humans
- Phase 2
  - *In vivo* studies
    - Acute airway injury/inflammation and metal and chemical constituent bioavailability will be assessed following intranasal or intratracheal sand exposure.

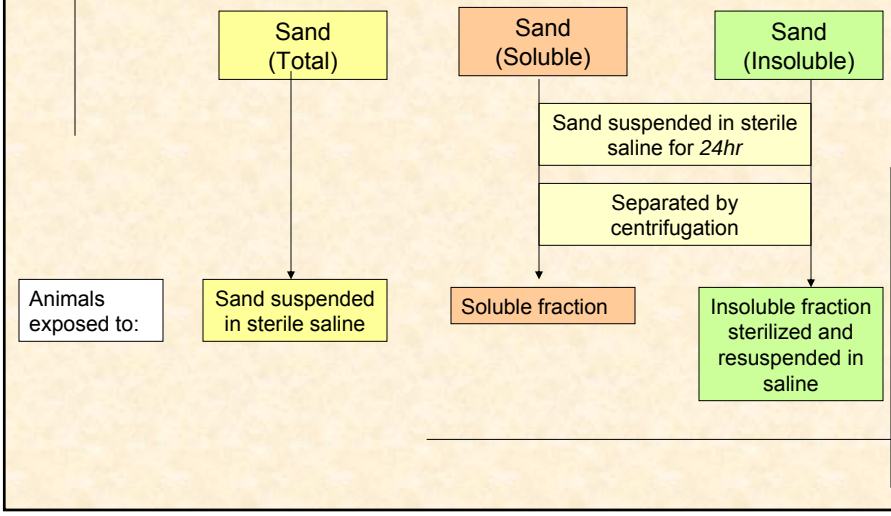
- **In Vitro Dissolution Studies of Sand**
  - Can provide quantitative information on the potential for release of significant amounts of chemicals from particles which may be deposited in the lung after inhalation *(Kanapilly et al., 1973)*
  - **2 Systems**
    - Lung Stimulant Fluid (LSF) pH ~ 7.4, represents the general lung environment *(Kanapilly et al., 1973)*
    - Phagolysozyme Stimulant Fluid (PSF) pH ~ 4.5-5, phagolysomes *(Stefaniak, 2005)*
      - "More realistic" in vivo scenario, inhaled particles deposited deep in the lung are phagocytized by alveolar macrophages and localized in lysozymes.

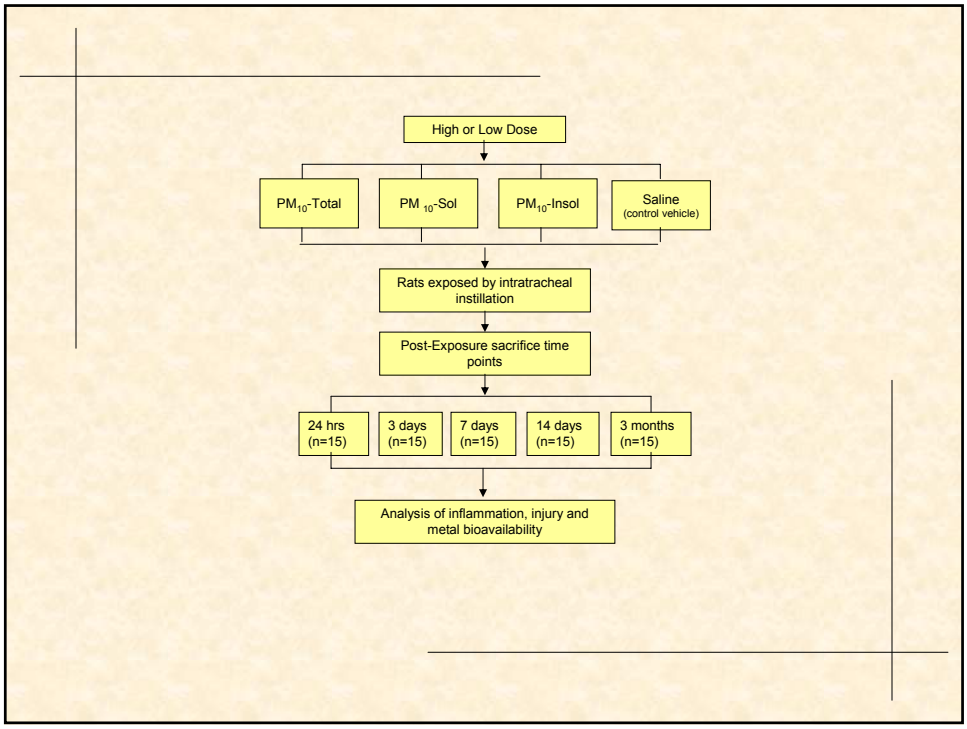
- **2 System Approach: LSF & PSF**
  - Methods of Hodgson et al. (2004)
  - Both systems maintained at 37 ºC
  - At various time points (1hr-14 days), samples will be removed from each dissolution system and assayed for metals and chemical constituents which may have leached off the particles.
  - Potential rate of in vivo leaching of chemicals will be calculated.
  - Mathematical lung deposition models will be employed to determine lung burden and potential health risk.

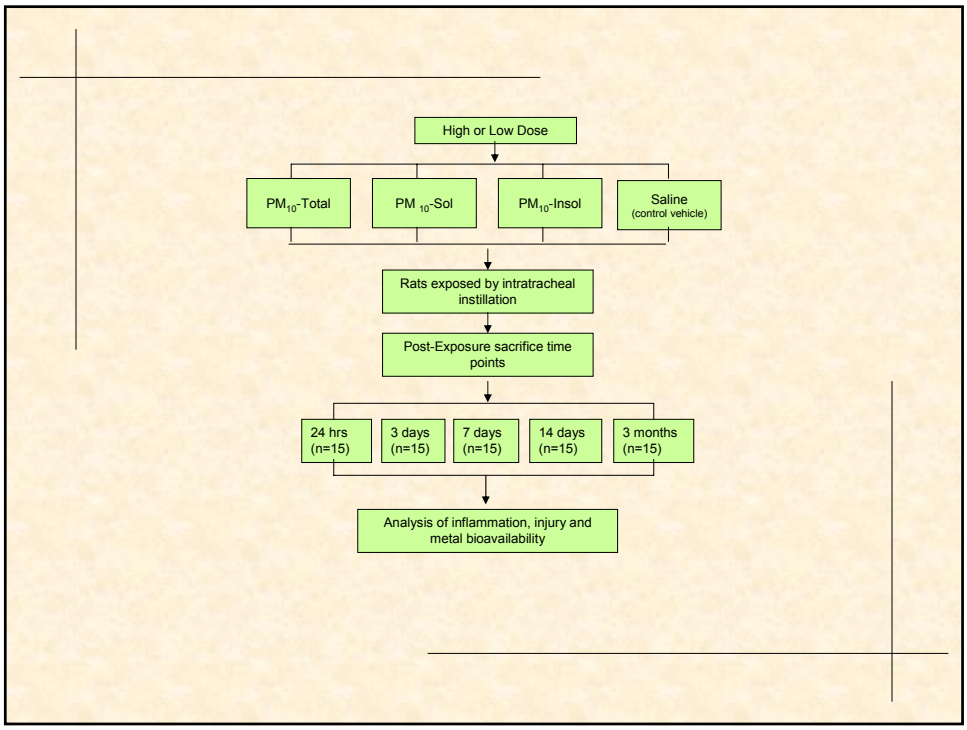
- **Upper and Lower Airway Sand Exposures**
  - Airborne particles less than 10 µm are respirable *(Ziskind et al., 1976)*
    - 5 µm or less can reach the alveoli
    - 1 µm or less have a high probability of being deposited in the alveoli *(Parkes 1983)*
  - Airborne particles as large as 100 µm can deposit in the nasal passages *(NCRP Report 125, 1997)*

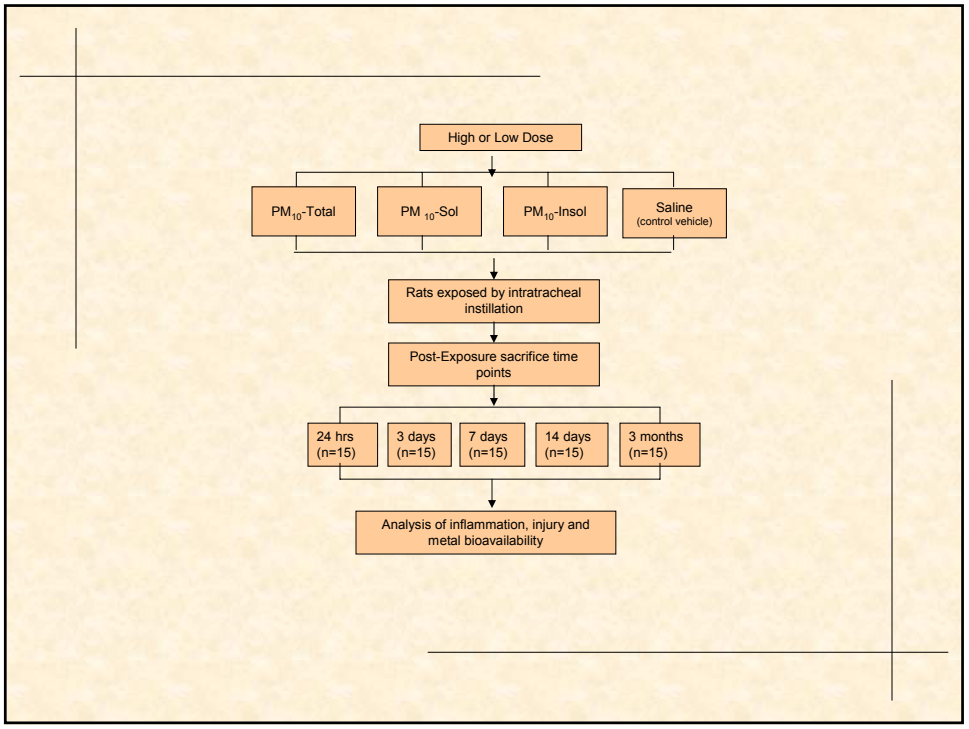
- Approach
  - Rat model
  - Intratracheal instillation (PM_{10})
    - Provides valuable mechanistic info which correlates will with inhalation exposures *(Henderson et al. 1995)*
  - Intranasal instillation (PM_{20} & PM_{40})
    - Alternative to nose-only inhalation; can be used to directly exposed the nasal cavity to various substances *(Lewis et al., 2005; Hendriksson et al., 1997)*

- Are the observed effects due to the sand, contaminants or both




High or Low Dose
PM10-Total
PM 10-Sol
PM10-Insol
Saline
(control vehicle)
Rats exposed by intratracheal instillation
Post-Exposure sacrifice time points
24 hrs (n=15)
3 days (n=15)
7 days (n=15)
14 days (n=15)
3 months (n=15)
Analysis of inflammation, injury and metal bioavailability


High or Low Dose
PM10-Total
PM 10-Sol
PM10-Insol
Saline
(control vehicle)
Rats exposed by intratracheal instillation
Post-Exposure sacrifice time points
24 hrs (n=15)
3 days (n=15)
7 days (n=15)
14 days (n=15)
3 months (n=15)
Analysis of inflammation, injury and metal bioavailability



- **Sacrifice**
  - Lung lavages
    - Leukocyte cell differentials
    - Total protein
    - Albumin
    - LDH
      - cytoplasmic enzyme released from damaged cells
    - β glucuronidase
      - lysosomal enzyme released by activated/damaged PMN/AM
    - Alkaline phosphatase
      - tissue damage & type II cell proliferation (fibrosis)
    - N-acetyl-β-D-glucosaminidase (NAG)
      - secreted by macrophages in response to phagocytosis of particulate matter
  - Nasal Lavages
    - Leukocyte cell differentials
    - Total Protein
    - Albumin
  - Cytokine profile
  - Histopathology
  - Major organs analyzed for metals and other chemical constituents

- Other studies?
  - Are studies actually needed? Is it better to start with an *in vitro* approach ?
  - More information from the field
    - Survey deployed troops
    - track respiratory complaints
  - The Department of Defense Serum Repository (DoDSR)
    - Started in 1989/ HIV testing
    - Army, Navy, Air Force
    - Stores ooperational deployment specimans
    - Serum markers of lung injury